JPW 1642

Dkt. 1884/43016-A-PCT-US/JPW/BJA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Philip O. Livingston and Friedhelm Helling

Serial No.: 08/196,154 Examiner: A. Holleran

Filed : November 16, 1995 Group Art Unit: 1642

For : GANGLIOSIDE-KLH CONJUGATE VACCINE PLUS QS-21

1185 Avenue of the Americas
New York, NY 10036
October 25, 2005

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

COMMUNICATION IN RESPONSE TO OCTOBER 14, 2005 NOTICE TO FILE CORRECTED APPLICATION PAPERS - NOTICE OF ALLOWANCE MAILED

This Communication is submitted in response to an October 14, 2005 Notice to File Corrected Application Papers issued in connection with the above-identified application. A response to the October 14, 2005 Notice is due November 13, 2005. Accordingly, this response is being timely filed.

The October 14, 2005 Notice to File Corrected Application Papers issued in connection with the above-identified application states that the subject application has been accorded an Allowance date and is being prepared for issuance. A copy of the October 14, 2005 Notice is attached hereto as **Exhibit A**.

The October 14, 2005 Notice further states that the application is incomplete because original pages 72-77 of the specification are missing. In response, applicants attach hereto as **Exhibit B**, original pages 72-77 as filed

in the original specification.

Applicants note that the subject application is a §371 National Stage of PCT International Application No. PCT/US94/00757, and that this application was received by the U.S. Patent and Trademark Office as the Receiving Office on January 21, 1994 with 143 pages of specification, 5 pages of claims, 1 page of abstract and 26 sheets of figures, i.e. containing pages 72-77, as evidenced by the returned, stamped postcard attached hereto as **Exhibit C**. Furthermore, PCT International Application No. PCT/US94/00757 was published as WO 94/16731 on August 4, 1994, and contains pages 72-77. Moreover, this application was transmitted to the International Bureau on February 25, 2005 (see PCT/RO/105 attached hereto as **Exhibit D**), and this is the application which entered the national stage as identified above. Applicants also note that no reference was made by the U.S. Patent Office during prosecution of the subject application to any missing original pages. Accordingly, it is applicants' understanding that missing pages 72-77 were filed as part of the original specification, were published as part of International Publication WO 94/16731, and that these pages have been mislaid at the U.S. Patent Office.

If a telephone conference would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee is deemed necessary in connection with the filing of this Communication. However, if an additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

10/25/05

John P. White Date
Reg. No. 28,678

1884 / 43016-A-PCT-US JPW

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Serial Number
08196154

Date Mailed
10/14/05

30 day Response Due: 11/13/05 (MAL)

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given 30 days from the mail date of this Notice within which to correct the informalities indicated below. A failure to reply will result in the application being ABANDONED. This period for reply is NOT extendable under 37 CFR 1.136 (a) or (b).

- Original pages 72-77 of the specification are missing.

APPLICANT MUST SUPPLY MISSING INFORMATION WITHIN 30 DAYS OF THE MAIL DATE OF THIS NOTICE.

A copy of this notice MUST be returned with the reply. Please address response to Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

Rori Burch
USPTO
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/196,154	11/16/1995	PHILIP O. LIVINGSTON	43016-A-PCT-	5954

7590 10/14/2005

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1185 AVENUE OF THE AMERICAS
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EXAMINER	
HOLLERAN, ANNE L	

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 10/14/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

To confirm the GD2 crossreactivity of IgG antibodies, postvaccination serum from patient No. 2 was preincubated with either GM2 or GD2 before performing the immune stain (Figure 8B). Reactivity with GM2, and with GD2 in the melanoma ganglioside extract, was completely inhibited by preincubation with GM2. On the other hand, preincubation of the same serum with GD2 resulted in inhibition of GD2 reactivity only, and did not change reactivity with GM2. These results suggest the presence of two populations of antibodies, one reacting with GM2 alone and another with reactivity for GM2 and GD2.

Subclass determination of IgG antibodies

High titer IgG sera from the six patients immunized with GM2-KLH and QS-21 were tested by ELISA using a panel of IgG-subclass specific secondary antibodies. The results are summarized in Table 4. The IgG antibodies in all six sera were of IgG1 and IgG3 subclass.

Complement-mediated cytotoxicity

Effector function of anti GM2 antibodies in the serum of patients vaccinated with GM2-KLH and QS-21 (diluted 1:5) was tested by complement-mediated cytotoxicity assays. As shown in Table 5, post-vaccination sera of all six patients lysed GM2-positive SK-MEL-173 melanoma cells in the presence of human complement. Prevaccination sera showed no cytotoxicity with addition of complement, and postvaccination sera were not cytotoxic with GM2 negative melanoma cells or when complement was not added. Clearly, these are only preliminary results, and more detailed study of cell surface binding and cytotoxic effector functions of vaccine-induced antibodies and their subclasses is now underway.

Discussion

In a series of studies in patients with malignant melanoma, the objective has been to construct vaccines that are effective in inducing production of antibodies against three gangliosides often overexpressed in melanoma - GM2, GD2 and GD3. The initial approach was to vaccinate patients with unconjugated gangliosides adsorbed to BCG. In this way we were able to induce antibody production against GM2 (5,6) but not GD2 or GD3. GM2 antibodies induced by GM2-BCG vaccines were mostly of the IgM class, the antibody response was of short duration, and booster immunization resulted again in a brief period of IgM antibody production similar to the primary response - all characteristics of a T-cell independent immune response, well known from studies of other carbohydrate antigens. Even so, vaccine-induced production of GM2 antibodies by patients with Stage III melanoma after surgery was associated with increased survival (6,7). This observation suggested that melanoma gangliosides are appropriate candidates for vaccine construction, and that melanoma ganglioside vaccines of increased immunogenicity might result in superior clinical outcomes. As the relevant epitopes of melanoma gangliosides are carbohydrates it is helpful to consider studies that have been aimed at increasing the immunogenicity of other carbohydrate vaccines, notably vaccines against certain bacterial infections.

The major distinction of the immune response to carbohydrate antigens, as opposed to protein antigens, is that it does not depend on the thymus. The concept that carbohydrate antigens are thymus-independent (TI) is based on the observation that neonatally thymectomized mice as well as thymic mice show unimpaired humoral immune responses to bacterial polysaccharides (15). B-cells that respond to TI

antigens show several characteristic features. They appear later in ontogeny, are long-lived, and do not require T-cells for activation, at least not in vivo. Although T-cells are required for B-cells to respond to TI antigens in vitro, the nature of the T-cell effect is poorly understood and clearly different from the MHC-restricted T-cell help in the T-dependent antibody response to protein antigens. While T-cells are not indispensable for the in vivo antibody response to TI antigens, antibody levels are higher when T-cells are present, suggesting a general augmenting activity of T-cells, again by unknown mechanisms (16).

A large variety of approaches has been explored in attempts to increase the immunogenicity of carbohydrate antigens. They include chemical modification (17), administration with adjuvants, non-covalent complexing with proteins, covalent attachment to immunogenic protein carriers (18), and replacement of the carbohydrate epitope by a protein replica, either peptides synthesized de novo (so-called mimotopes, 19) or anti-idiotypic antibodies (20). Most of these approaches result in increased T cell help for the carbohydrate specific antibody response. While each has shown promise in initial experimentation, covalent attachment of carbohydrate antigens to immunogenic T-dependent protein carriers, as first suggested for haptens (21) and then disaccharides (22), is the concept that has been pursued most vigorously, resulting in vaccines that have in some instances been shown to be highly effective in recent clinical trials.

Excellent examples are H. influenzae type b (Hib) polysaccharide protein conjugate vaccines. Four vaccines that have been developed over the last decade differ in the carbohydrate components, protein carriers and linkers

between carbohydrate and protein (23-27). In comparative studies in children, conjugate vaccines induced a much stronger antibody response than unconjugated Hib phosphoribosyl/ribitolphosphate polysaccharide (PRP) vaccine (28). Of particular interest are observations that young children first immunized with HbOC (oligosaccharide-nontoxic diphtheria toxin) or PRP-OMPC (outer membrane protein complex of Neisseria meningitidis type B) vaccines and later challenged with unconjugated PRP vaccine showed an anamnestic IgG response even if challenged at an age at which they do not respond to primary immunization with the unconjugated vaccine (29,30). How T-cells are engaged, and how they interact with Hib PRP-responsive B-cells, is still far from clear. The fact that increased immunogenicity and T-dependence require a covalent bond between PRP and protein suggests that the proximity between protein and PRP must not be disturbed, at least not in the early phase of antigen processing. As the isotype and biological activities of antibodies induced by Hib PRP and Hib PRP conjugates are the same, it appears that the B-cells that respond to the conjugate-induced T-cell signal are qualitatively identical with those engaged by Hib PRP alone.

Drawing on the substantial experience that has accumulated in the development of carbohydrate vaccines for bacterial infections, applicants have explored, over the past several years, similar approaches in attempts to increase the immunogenicity of melanoma gangliosides. Chemical modification of GD3, resulting in amide, lactone or gangliosidol formation, or O-acetylation, produced derivatives that were highly effective in inducing antibody production in patients with melanoma. However, the antibodies induced by these GD3 derivatives did not cross-react with GD3 (31,32). An anti-idiotypic antibody BEC-2, mimicking GD3, has been developed by immunizing mice

with the monoclonal antibody R24 which recognizes GD3. Rabbits immunized with BEC-2 produced anti-GD3 antibodies (33), and initial studies of the immunogenicity of BEC-2 in human patients are underway.

Regarding conjugate vaccines, initial studies with GD3 in the mouse were concerned with three issues - development of the conjugation method, selection of the carrier protein, and choice of the adjuvant (7). The optimal conjugation procedure involved ozone cleavage of the double bond of GD3 in the ceramide backbone, introduction of an aldehyde group, and coupling to protein aminolysyl groups by reductive amination. Of five carriers tested - poly-l-lysine, keyhole limpet hemocyanin (KLH), cationized bovine serum albumin, Neisseria meningitidis outer membrane protein complex (OMPC), and multiple antigenic peptide constructs containing four repeats of a malarial T-cell epitope on a branching polylysine core -, KLH was found to be most effective. Noncovalent GD3/KLH complexes were not immunogenic. The best adjuvant was QS-21, a homogeneous saponin fraction purified from the bark of Quillaja saponaria Molina. The characteristics of the antibody response to immunization with GD3-KLH conjugate and QS-21 included a) a high initial IgM antibody titer, b) a rapid secondary rise of IgM antibody titers after booster immunizations, c) maintenance of IgM antibody titers after booster immunization for up to ten weeks, and d) consistent production of IgG antibodies at high titers, parallel to IgM antibody production except for the initial delay of two weeks. These findings have now been reproduced in human melanoma patients by immunization with another ganglioside conjugate vaccine, GM2-KLH, using the same conjugation procedure. As in the mouse studies, QS-21 proved to be a significantly more effective adjuvant than DETOX or BCG, with acceptable toxicity.

The GM2 antibody response had many characteristics of a T cell dependent response. It was long-lasting, and antibodies of IgG1 and IgG3 subclass (usually associated with a T cell dependent immune response) were induced. As seen with the Hib-PRP vaccines, these isotypes were the same as those induced occasionally at low titers with unconjugated GM2/BCG vaccines. The lack of a clear booster effect in the sustained high-titer IgM and IgG response after vaccinations three and five months following the initial series may be explained by the fact that the patients were immunized at two-week intervals initially. In the classical experiment showing the secondary response to protein antigens, the second injection of antigen is given four weeks after the first. Antibody levels after the first immunization are higher between one and two weeks after the injection, and then decline to very low levels before the booster injection is given after four weeks. In the immunization schedule applicants chose, the initial antibody response did not subside but increased in a stepwise fashion in response to the first four vaccinations at two-week intervals, anticipating the secondary response that is seen in a more dramatic fashion in the classical experiment. Unlike the antibody response to most protein antigens, the IgM response was long-lasting, and IgM antibodies remained at higher titer than IgG antibodies, even after repeated booster immunizations, as is characteristic for carbohydrate antigens. Hence the immune response against gangliosides which contain a comparably short oligosaccharide chain linked to a lipid backbone and which are autoantigens show much in common with the immune response against Hib-PRP and other bacterial carbohydrates.

The development of the GM2-conjugate vaccine will make it possible to determine whether higher levels of IgM and IgG antibodies against GM2, sustained over longer periods, will

PCT/US 94/00757

Applicant Sloan-Kettering Institute for Cancer Research

Client Sloan(1747) File No. 43016-A-PCT Atty. JPW

Date 21 January 1994

Kindly acknowledge receipt of the accompanying New International Patent Application Under the Patent Cooperation Treaty corresponding to Philip O. Livingston and Friedhelm Helling for GANGLIOSIDE-KLH CONJUGATE VACCINES PLUS QS-21, designating EP, AU, CA, FI, HU, JP, KR, NO, NZ, RU and US (continuation-in-part), including Specification (143pp), Claims(5pp), Abstract(1p), Figures(26pp), Transmittal Letter, PCT Request Form(4pp), Fee Calculation Sheet, a check for $3,910.00, an Express Mail Certificate bearing Label No. IB535735123US, dated 21 January 1994, Form PTO-1595 in duplicate and original signed Assignment.

by placing your receiving date stamp hereon and returning to us.

52 Rec'd PCT/ 21 JAN 1994

Applicants: Philip O. Livingston et al.
U.S. Serial No.: 08/196,154
Filed: October 25, 2005
Exhibit C

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

JOHN P. WHITE
COOPER & DUNHAM
30 ROCKEFELLER PLAZA
NEW YORK, NEW YORK 10112

PCT

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing (*day/month/year*)	25 FEB 1994
Applicant's or agent's file reference: 43016-A-PCT	IMPORTANT NOTIFICATION

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US94/00757	21 JAN 94	22 JAN 93

Applicant: SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH

Title of the invention: GANGLIOSIDE-KLH CONJUGATE VACCINES PLUS QS-21

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

☑ was transmitted to the International Bureau on 25 FEB 1994.

☐ has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:

☐ because the necessary national security clearance has not yet been obtained.

☐ because (*reason to be specified*):

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

3. FOREIGN TRANSMITTAL LICENSE INFORMATION Completed by: [illegible] 9407

☐ Additional license for foreign transmittal not required. This subject matter is covered by a license already granted on the equivalent U.S. national application. Refer to that license for information concerning its scope.

☐ License for foreign transmittal not required. 37 CFR 5.11(e)(1) or 37 CFR 5.11(e)(2). However, a license may be required for additional subject matter. See 37 CFR 5.15(b).

☑ Foreign transmittal license granted. 35 U.S.C. 184; 37 CFR 5.11 on _____ (date):

☑ 37 CFR 5.15(a) ☐ 37 CFR 5.15(b)

Name and mailing address of the receiving Office: COMMISSIONER OF PATENTS AND TRADEMARKS, Box PCT, Washington, D.C. 20231, Attn: RO/US	Authorized officer: YVETTE E. SIMMS, INTERNATIONAL DIVISION
Facsimile No.	Telephone No.

Form PCT/RO/105 (July 1992)

Applicants: Philip O. Livingston et al.
U.S. Serial No.: 08/196,154
Filed: October 25, 2005
Exhibit D